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Microbial Metabolism and Dynamic Changes in the Electrical Conductivity of Soil Solutions: a Method for Detecting Extraterrestrial Life[1]

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The addition of 0.5% glucose solutions to 12 different air-dried soils always resulted in increased electrical conductivity and water-soluble Ca and Mg in the soil solutions. The kinetics and magnitude of these changes for at least two and usually all three of these parameters over a 14-day period were clearly distinguishable from the changes in heat-sterilized controls or unsterilized controls without added glucose. In general, maximal values were achieved more rapidly under aerobic than anaerobic incubation. Some soils (less than half) also showed significant increases in water-soluble Na or K when compared with the controls. The 12 different soils studied represented four general soil groups: I, leached acid upland soils; II, saline alkaline soils; III, nonsaline neutral soils; and IV, high organic soils. Viable counts ranged from 10^4 to 10^7 per cm^3 of air-dried soil. Glucose metabolism by the indigenous soil microbiota was always accompanied by a significant decrease in the pH of soil solutions, but not necessarily by an increase in the viable count. The feasibility of using electrical conductivity and water-soluble Ca and Mg measurements to detect metabolic activity, either alone or in conjunction with other life detection techniques, is discussed.

Any analysis of the problem of detecting extraterrestrial life resolves itself ultimately into the question of how one defines a living system. Young et al. (16) considered the problem from the biologist's point of view and concluded that living systems would have in common the attributes of (i) a carbon-based macromolecular chemical composition; (ii) the ability to grow and reproduce; and (iii) the ability to utilize materials and energy through the process of metabolism. Although many life detection experiments have been proposed, all fall within one or more of these categories (reviewed in 2, 6, 8, 10, 15, 16).

Four experiments bearing on the detection of life have been selected by the National Aeronautics and Space Administration for the 1975 Viking mission to Mars (4, 9, 12). In one experiment Martian soil will be analyzed for volatile and pyrolyzed organic compounds by gas chromatography-mass spectrometry (1). The remaining three experiments are designed to detect metabolic activity in Martian soil. One experiment measures photosynthetic and dark fixation of ^{14}C-labeled CO_2 and CO under relatively dry conditions (3); the second measures the release of $^{14}CO_2$ from a dilute aqueous solution of ^{14}C-labeled organic compounds added to soil (5); the third measures the production or consumption of H_2, N_2, O_2, CH_4, and CO_2 by soil incubated with a complex, aqueous, organic medium (7).

From an ecological point of view, all living systems interact with the gas, liquid, and solid phases of the environment, but the three Viking metabolic experiments (3, 5, 7) measure only interaction with the gas phase, as does the unified approach proposed by Radmer and Kok (11) for subsequent Viking missions. If one assumes that water is as essential for life on Mars as on Earth, then Martian metabolic processes should also interact with the aqueous phase of the environment. As nutrients are converted into lower-molecular-weight metabolic intermediates and organic or mineralized end products, there should be a net increase in the number of ionic and electrically charged organic and inorganic molecules in solution and a corresponding increase in the electrical conductivity (decrease in impedance) of the aqueous phase of the environment. Dynamic changes in the impedance of the liquid phase as a

[1] Dedicated to the late Wolf Vishniac, one of the pioneers of exobiology, who died in the Antarctic in December 1973.

function of growth and metabolism have been demonstrated recently for pure cultures of bacteria (P. Cady, Int. Congr. Bacteriol., 1st, Jerusalem, Abstr. N-1, 1973); for bacteria in blood cultures (W. K. Hadley, G. Senyk, R. Michaels, and J. Seman, Abstr. Annu. Meet. Amer. Soc. Microbiol., Abst. M281, 1974); and for beer-spoiling bacteria (P. Lawless, J. Shaw, and S. J. Kraeger, Abstr. Annu. Meet. Amer. Soc. Microbiol., Abstr. E42, 1974). An automated system for impedance measurements in bacteriology has been described (P. Cady and S. W. Dufour, Abstr. Annu. Meet. Amer. Soc. Microbiol., Abstr. E43, 1974).

This paper reports the results of studies on dynamic changes in the electrical conductivity and other parameters of soil solutions as a function of metabolism by the indigenous microbiota and on the feasibility of their use as metabolic probes for detecting extraterrestrial life.

MATERIALS AND METHODS

Aerobic studies. Thirty cubic centimeters of soil and 80 ml of liquid in 300-ml Erlenmeyer flasks were incubated in the dark at 30 C without agitation. Ten-milliliter portions were removed and centrifuged in 15-ml Corex centrifuge tubes for 10 min at 10,000 × *g*. The supernatant fluid was filtered through a 0.22-μm membrane filter, and the filtrate was used for pH and electrical conductivity measurements as well as for analyses of water-soluble Na, K, Ca, and Mg.

Three different flasks were used for each soil. One contained 30 cm^3 of soil and 80 ml of filter-sterilized 0.5% glucose solution. A second contained 30 cm^3 of soil and 80 ml of sterile water. The third contained 30 cm^3 of dry heat-sterilized soil (160 to 170 C, 94 to 96 h) and 80 ml of sterile 0.5% glucose solution. The soils were dispensed by equal volumes rather than equal weights to conform more closely to actual practice on the Viking Mars mission (4).

Anaerobic studies. Three cubic centimeters of soil and 8 ml of liquid were added to 15-ml Corex centrifuge tubes capped with Morton stainless steel closures. The tubes were incubated at 30 C in the dark under $H_2 + CO_2$ in GasPak system anaerobic containers (Bioquest). Three sets of tubes were used for each soil studied. One set contained unsterilized soil plus filter-sterilized 0.5% glucose solution; one contained unsterilized soil and sterile water; and one contained dry heat-sterilized soil (160 to 170 C, 96 h) and filter-sterilized 0.5% glucose solution. One tube was taken from each set on days when measurements were to be made. One milliliter was removed from each tube and incubated in thioglycolate broth (Difco) for viability and sterility checks. The remainder of the tube contents was then centrifuged and treated as for the aerobic studies.

Viable counts. Aerobic plate counts were made by taking 1-ml portions from the aerobic flasks, making decimal dilutions in dilute Trypticase soy broth (BBL, 1 g/liter), spreading 0.1 ml of the decimal dilutions on the surface of dilute Trypticase soy agar plates in triplicate, and incubating the plates at 30 C for 7 days. Viable aerobic counts are expressed as colony-forming units per ml of soil-liquid mixture, except for the counts in Table 1, which are expressed as colony-forming units per cubic centimeter of air-dried soil.

Soils. Twelve different soils were studied. All were provided by the University of California, Davis, except Death Valley 1S and Yolo, which were collected by E. L. Merek of our laboratory. All soils were collected from the upper 30 cm, air-dried, sieved through 0.25-inch (about 0.8 cm) mesh, and mixed before being stored in glass jars. The soils are divided into four different groups: leached, acid upland soils (group I), saline alkaline soils (group II), nonsaline neutral soils (group III), and high organic soils (group IV). Descriptions and analyses for each soil are given in Tables 1 and 2.

Analytical. The electrical conductivity of the soil solutions was measured at ambient temperature (22 ± 1 C) by using a Beckman Instruments conductivity cell with platinum electrodes (no. G10Y184) in conjunction with a model RC16B2 conductivity bridge from Industrial Instruments, Inc. Water-soluble Na, K, Ca, and Mg were measured in appropriate dilutions with a Perkin-Elmer model 303 atomic absorption spectrometer.

RESULTS

Both aerobic and anaerobic experiments were conducted and gave reproducible results when repeated. The results of the anaerobic experiments are emphasized because oxygen in the Martian atmosphere is either absent or below the level of detection. Under anaerobic incubation, significant changes occurred in the electrical conductivity of soil solutions from group I soils when supplemented with glucose. These soil solutions, with low initial electrical conductivity (Table 2), showed much greater changes with time in electrical conductivity than control solutions from sterile soil supplemented with glucose or unsterilized soil with only water added. Representative results with Siskiyou soil are illustrated in Fig. 1. Solutions from group III and IV soils, with somewhat higher initial electrical conductivity, also showed significant changes in electrical conductivity when compared with the controls. Typical results with Ramona (group III) and Staten (group IV) soils are shown in Fig. 2 and 3, respectively. A more severe challenge to the use of electrical conductivity measurements to detect metabolic activity was presented by the saline alkaline soils from group II, with sizeable quantities of extractable cations and relatively high initial electrical conductivity (Table 2). Metabolic activity was easily detectable with Waukena

TABLE 1. *Description[a] of soils*

Group	Soil	Description	Bulk density (g/cm³)
I. Leached acid upland soils	Siskiyou	Gray-brown podzolic	1.471
	Aiken	Red podzolic	1.053
II. Saline alkaline soils	Waukena	Calcic brown solonchak	1.354
	Holtville	Red desert alluvial	1.269
	Death Valley 1S	Undescribed desert	1.453
III. Nonsaline neutral soils	Yolo	Noncalcic brown alluvial	1.323
	Ramona	Noncalcic brown	1.429
	Salinas	Calcic brown alluvial	1.259
	Hesperia	Calcic brown alluvial	1.355
	Panoche	Gray desert alluvial	1.315
IV. High organic soils	Staten	Prairie bog	0.696
	Tule Lake	Undescribed diatomaceous	0.558

[a] All the soils except Death Valley 1S and Tule Lake are described by Storie and Weir (13).

TABLE 2. *Analysis[a] of soils*

Soil	EC[b] (μmho/cm)	pH as paste	Extractable cations[c] (μg/g)				Organic C (μg/g)	Total N (μg/g)	Viable counts[d]
			Na	K	Ca	Mg			
Siskiyou	6	6.30	1	165	210	90	4,910	340	2.33×10^6
Aiken	17	4.85	69	165	100	120	8,170	650	6.57×10^5
Waukena	2,200	10.30	4,800	84	1,320	75	940	187	3.64×10^4
Holtville	2,210	8.05	2,070	1,155	1,770	90	3,520	745	2.68×10^7
Death Valley IS	750	8.45	1,365	485	5,030	84	420	75	2.13×10^4
Yolo	79	7.10	63	150	250	315	9,670	1,060	3.41×10^7
Ramona	31	7.60	36	270	840	75	5,710	628	5.65×10^6
Salinas	105	7.55	234	735	3,950	105	8,170	1,565	6.35×10^7
Hesperia	54	7.00	150	165	660	66	2,530	251	5.84×10^6
Panoche	240	7.85	18	345	2,540	90	3,750	560	1.81×10^7
Staten	140	5.85	306	120	3,620	60	210,000	13,435	1.09×10^7
Tule Lake	360	7.85	520	720	6,400	360	27,530	5,005	1.22×10^6

[a] All analyses except viable count performed by Soil Control Laboratory, Watsonville, Calif.
[b] Electrical conductivity, 1:5 solution.
[c] Extracted by electrodialysis in 0.05 N boric acid.
[d] Expressed as colony-forming units (CFU) per cm³ of soil.

and Death Valley 1S soils within 7 days (Fig. 4). With Holtville, detection of metabolic activity was more equivocal. It took 11 days before the electrical conductivity of the glucose-supplemented solution exceeded that of the controls by approximately 1,000 μmho/cm. When incubation was aerobic rather than anaerobic, changes in the electrical conductivity of glucose-supplemented solutions from all the soils, including Holtville (Fig. 5), were clearly distinguishable from the changes observed in the controls.

Changes in the electrical conductivity of soil solutions resulting from the metabolism of glucose by the indigenous microbiota were also accompanied by dynamic changes in the water-soluble Ca and Mg for all 12 soils, whether incubated anaerobically or aerobically. The one exception was Waukena, incubated aerobically, which did not show a comparable increase in water-soluble Mg. Representative results of only the anaerobic experiments are shown in Fig. 6 for Ca with Ramona soil (group III) and Fig. 7 for Mg with Death Valley 1S soil (group II). Comparable results were not always observed for water-soluble Na and K under aerobic and anaerobic incubation. Less than half the 12 soils tested yielded solutions that showed significant increases in these elements when compared with the controls.

Metabolism of glucose by the indigenous microflora always resulted in a decrease with time of from 1 to 2 pH units compared with the controls, regardless of the initial pH, the soil tested, or whether incubation was aerobic or anaerobic. This is illustrated in Fig. 8 for representative soil solutions from the four soil

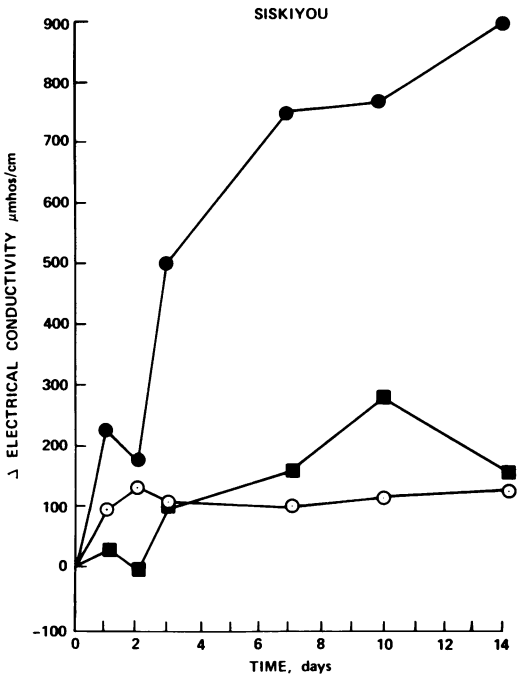

FIG. 1. *Changes in the initial electrical conductivity of soil solutions from Siskiyou (group I) soil incubated anaerobically. Symbols:* (●) *soil + 0.5% glucose;* (⊙) *sterile soil + 0.5% glucose;* (■) *soil + water. The initial electrical conductivity was:* (●) *39;* (⊙) *452;* (■) *35.*

groups under anaerobic incubation.

Glucose metabolism in soil did not necessarily result in a greater increase in viable counts compared with the counts in soil with no added glucose. Comparative plate counts for representative soils from each of the four soil groups incubated aerobically are shown in Fig. 9. Although glucose metabolism resulted in a greater increase in viable counts in Death Valley 1S and Tule Lake soils, it resulted in a decrease in counts in Siskiyou soil after the first day and appeared to have no significant influence on the counts in Panoche soil throughout the 14 days of the experiment. All the heat-sterilized soils remained sterile and all the unsterilized soils contained viable microorganisms as determined by plate counts in the aerobic experiments and by incubation in thioglycolate broth in the anaerobic experiments.

DISCUSSION

The soil with its resident population of microorganisms, indigenous carbon, nitrogen, and minerals can be considered a relatively stable ecosystem. When perturbed, the system will respond by establishing a new state of physical, chemical, and biological equilibrium that may or may not be similar to the previous state. One approach to detecting life in soil is to stimulate the system so that the biological component of the system is selectively perturbed and a metabolic response is induced.

Our results indicate that, for the parameters we have measured (electrical conductivity, water-soluble Na, K, Ca, and Mg, and pH), the addition of only water perturbed unsterilized soil, but the kinetics and extent of the responses over a period of 14 days were not sufficiently different from the sterilized soil control to allow the conclusion that metabolic activity was taking place. Evidently, metabolism of the indigenous carbon and energy sources in unsterilized soil contributed very little to the overall changes in the parameters measured, whatever the range of organic carbon in the soils (Table 2).

Selective stimulation of metabolism required the addition of an exogenous carbon and energy source. Glucose was chosen as the supplement because as a nonionic compound it had no effect as such on the zero-time electrical conductivity and because we expected it to be metabolized by at least some members of the indigenous

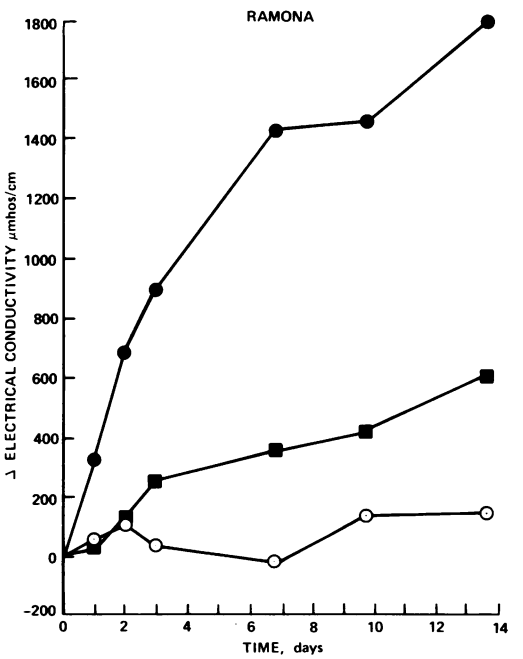

FIG. 2. *Changes in the initial electrical conductivity of soil solutions from Ramona (group III) soil incubated anaerobically. Symbols as in Fig. 1. The initial electrical conductivity was:* (●) *56;* (⊙) *494;* (■) *57.*

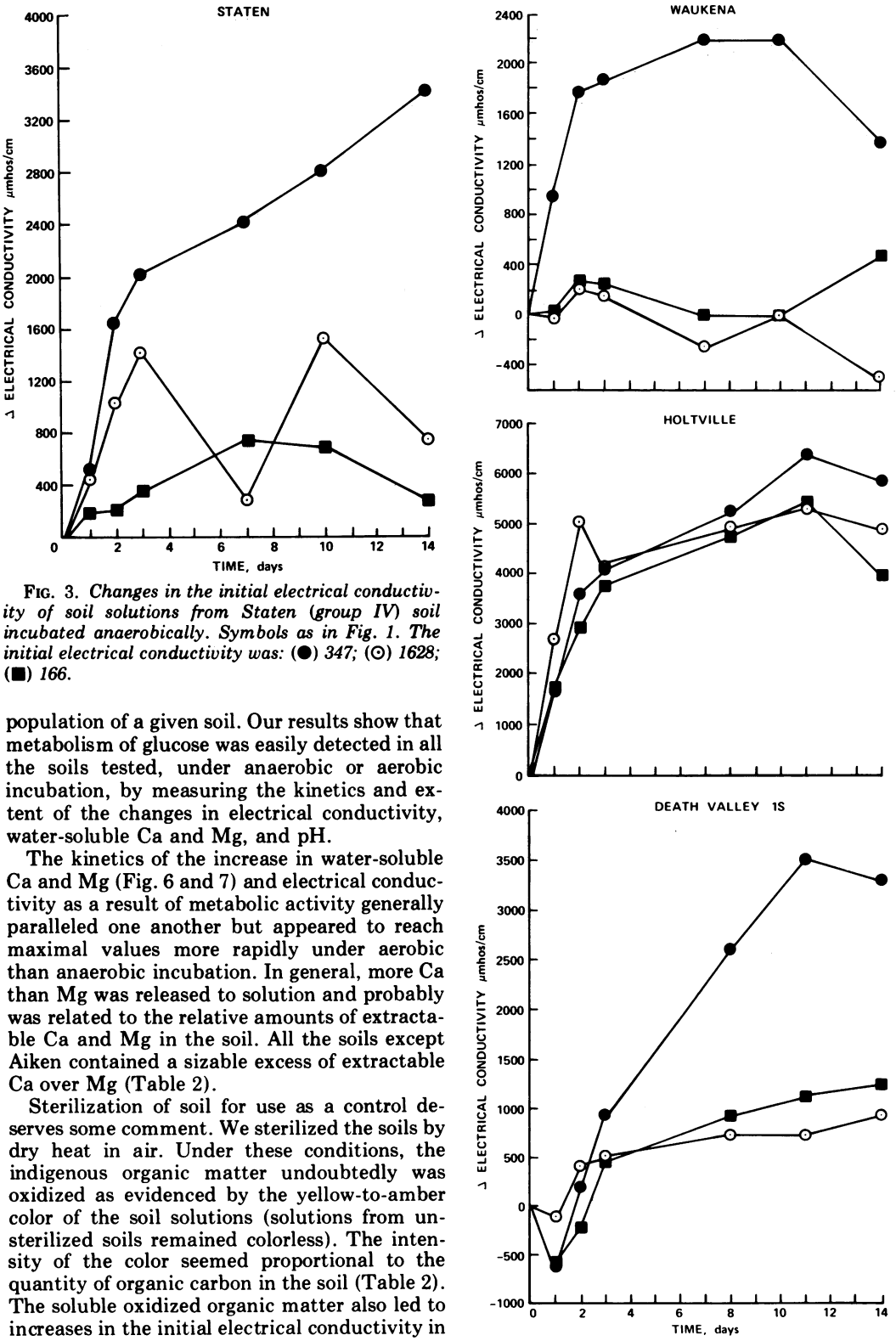

FIG. 3. *Changes in the initial electrical conductivity of soil solutions from Staten (group IV) soil incubated anaerobically. Symbols as in Fig. 1. The initial electrical conductivity was:* (●) *347;* (⊙) *1628;* (■) *166.*

population of a given soil. Our results show that metabolism of glucose was easily detected in all the soils tested, under anaerobic or aerobic incubation, by measuring the kinetics and extent of the changes in electrical conductivity, water-soluble Ca and Mg, and pH.

The kinetics of the increase in water-soluble Ca and Mg (Fig. 6 and 7) and electrical conductivity as a result of metabolic activity generally paralleled one another but appeared to reach maximal values more rapidly under aerobic than anaerobic incubation. In general, more Ca than Mg was released to solution and probably was related to the relative amounts of extractable Ca and Mg in the soil. All the soils except Aiken contained a sizable excess of extractable Ca over Mg (Table 2).

Sterilization of soil for use as a control deserves some comment. We sterilized the soils by dry heat in air. Under these conditions, the indigenous organic matter undoubtedly was oxidized as evidenced by the yellow-to-amber color of the soil solutions (solutions from unsterilized soils remained colorless). The intensity of the color seemed proportional to the quantity of organic carbon in the soil (Table 2). The soluble oxidized organic matter also led to increases in the initial electrical conductivity in

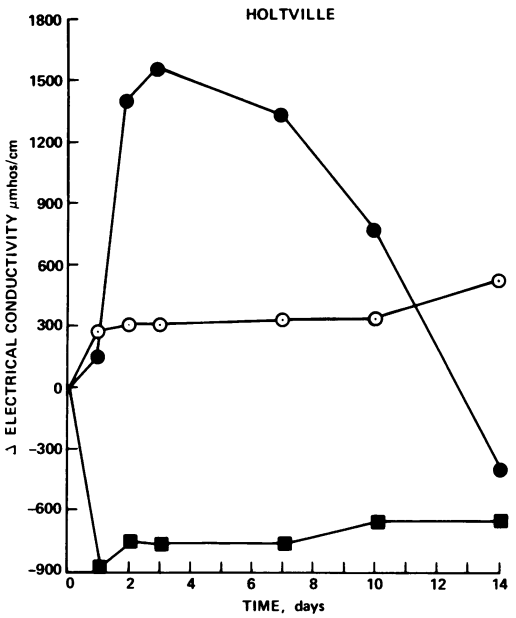

FIG. 5. *Changes in the initial electrical conductivity of soil solutions from Holtville (group II) soil incubated aerobically. Symbols as in Fig. 1. The initial electrical conductivity was: (●) 6100; (⊙) 5680; (■) 6870.*

most of the soils (Fig. 1-5), some decreases in the initial pH (Fig. 8), and an increase in initial water-soluble Ca (Fig. 6). These effects did not interfere with the subsequent kinetics and our ability to clearly distinguish microbial metabolism in unsterilized soils amended with glucose whose viable counts ranged from 10^4 to 10^7 per cm^3 of soil (Table 2). We do not know whether heat-sterilized controls would interfere with the detection of metabolic activity in soils harboring smaller populations. If so, then eliminating heat sterilization of soil as a control might be considered since soils with only water added appear to be as good, if not better, controls.

There are a number of advantages to measuring metabolism by electrical conductivity from the standpoint of a life detection experiment on Mars. (i) The method is nonspecific; i.e., it does not depend on the detection of a specific metabolite but responds to increases or decreases in the concentration of all electrically charged molecules and ions in solution, both organic and inorganic. (ii) The measurements are nondestructive and could be made continuously or at any convenient time interval, thereby enabling the kinetics of the process to be followed. (iii) Measurements can be made over a wide dynamic range, at least three decades in the 12 soils tested despite low or relatively high initial values (Table 1 and Fig. 1-5). (iv) No new technology is necessary. It should be relatively simple to insert electrodes in a cuvette assembly containing Martian regolith and an aqueous solution or to incorporate the electrodes as an integral part of the cuvette itself, as already demonstrated in a commercial instrument

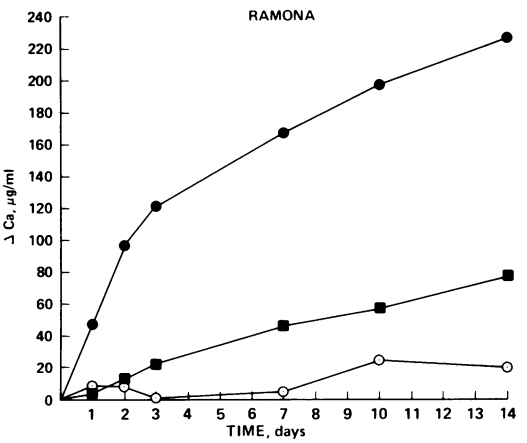

FIG. 6. *Changes in the initial water-soluble Ca of soil solutions from Ramona (group III) soil incubated anaerobically. Symbols as in Fig. 1. The initial water-soluble Ca (in micrograms per milliliter) was: (●) 3.3; (⊙) 65.4; (■) 2.2.*

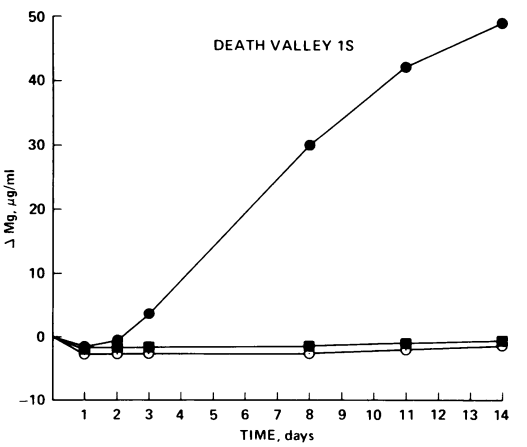

FIG. 7. *Changes in the initial water-soluble Mg of soil solutions from Death Valley 1S (group II) soil incubated anaerobically. Symbols as in Fig. 1. The initial water-soluble Mg (in micrograms per milliliter) was: (●) 2.4; (⊙) 3.7; (■) 3.1.*

←FIG. 4. *Changes in the initial electrical conductivity of soil solutions from Waukena, Holtville, and Death Valley 1S (group II) soils incubated anaerobically. Symbols as in Fig. 1. The initial electrical conductivity was: (●) 3045, (⊙) 3570, and (■) 3623 for Waukena; (●) 987, (○) 682, and (■) 630 for Holtville; (●) 2362, (○) 2730, and (■) 2310 for Death Valley 1S.*

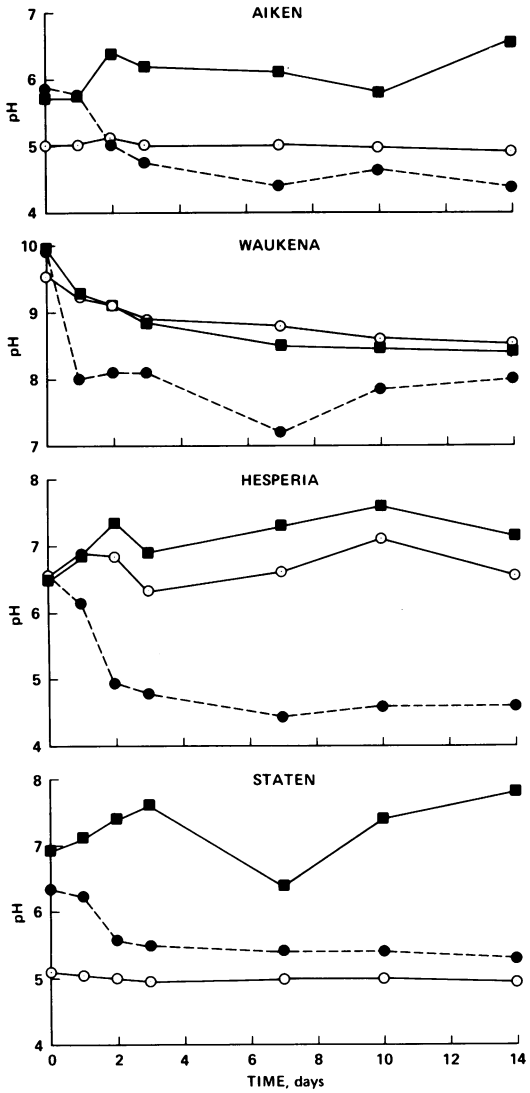

FIG. 8. *Changes in the initial pH of soil solutions from Aiken (group I), Waukena (group II), Hesperia (group III), and Staten (group IV) soils incubated anaerobically. Symbols as in Fig. 1.*

(Bactomatic, Inc., Palo Alto, Calif.). (v) A measurement of some kind is always obtained. In the absence of life or organic matter in the sample, at least some indication of the solubility and ionic nature of the Martian regolith would be obtained. Information of this kind would aid geochemists and cosmologists in understanding the evolution of the planet and its aqueous history.

Many, but not all, of the advantages noted above for electrical conductivity measurements also hold for measuring metabolic activity by dynamic changes in water-soluble Ca and Mg. Ion-specific electrodes are available for measuring water-soluble Ca and Mg, they can make continuous, nondestructive measurements over a wide dynamic range, and, even in the absence of life, they would almost certainly yield some information on the geochemical nature of the Martin regolith. However, ion-specific Ca and Mg electrodes are sensitive to only the ionic forms of these elements, and our experiments do not indicate what proportion of the water-solu-

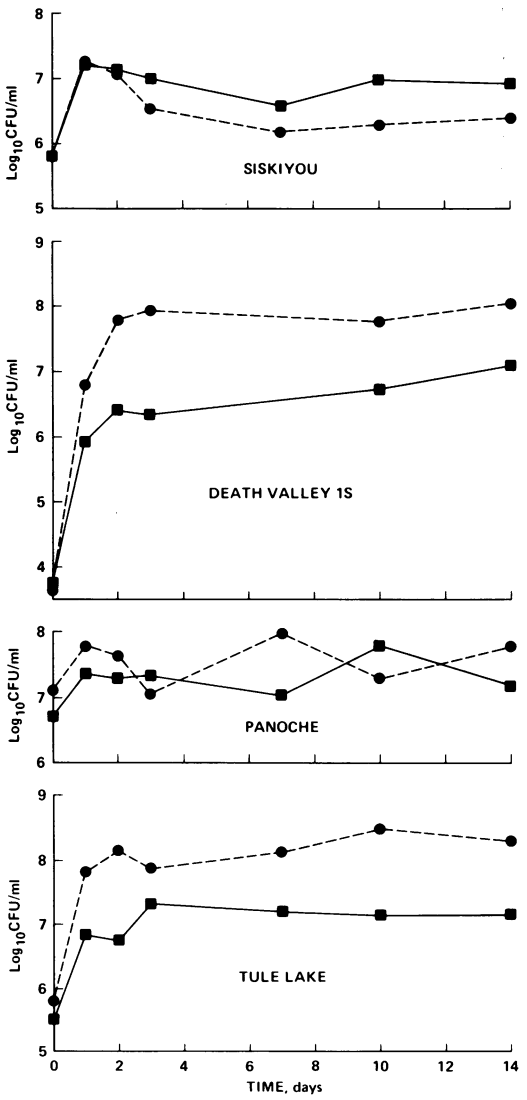

FIG. 9. *Changes in the viable count of Siskiyou (group I), Death Valley 1S (group II), Panoche (group III), and Tule Lake (group IV) soils incubated aerobically. Symbols as in Fig. 1; CFU, colony-forming units.*

ble Ca and Mg was released in ionic form as opposed to undissociated inorganic or organic salts or chelates. Alternatively, it may be possible to adapt the X-ray fluorescence spectrometer on the Viking Mars lander (14) for use in inorganic analyses of water-soluble inorganic matter released in metabolic experiments.

We conclude that measuring metabolic activity in soil solutions by means of dynamic changes in (i) the electrical conductivity, (ii) water-soluble Ca, or (iii) water-soluble Mg are feasible life detection methods. They represent alternatives to measuring metabolic activity in the gas phase for life detection (3-5, 7, 11) and can stand on their own as individual life detection experiments. They also demonstrate that the results of any life detection experiment, whether positive or negative, gain credibility in proportion to the number of different independent measurements made on the same sample. If any of the 12 soils we tested had been extraterrestrial in origin, the positive results obtained simultaneously for two or more of the three different parameters (electrical conductivity, water-soluble Ca, and water-soluble Mg) would lend enormous confidence to the conclusion that life was present.

The nondestructive nature of electrical conductivity and water-soluble Ca or Mg measurements also commends one or more of these for use in conjunction with other life detection techniques, especially those that are destructive of sample or are restricted to only one measurement, e.g., analyses for proteins, nucleic acids, D- or L-amino acids, specific enzymes, etc. The experimenter in such cases must decide, often arbitrarily, when to perform his experiment. If he could choose the optimal time to perform his experiment based on dynamic evidence of metabolic activity, his chances of achieving a significant result would be greatly enhanced.

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